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Optimizing The Use of Biological Mass of BEESPINE in Feeding Broiler Chickens

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Abstract: The aim of the present study was to evaluate the impact of incorporating 5% of beespine (biological bee biomass) into the diet of broiler chickens on the excretion and accumulation of lead (Pb) and overall poultry productivity. A total of 100 Cobb-500 broiler chickens were divided into control and experimental groups. The experimental diet consisted of 95% standard feed and 5% powdered beespine, pre-treated for increased bioavailability. Atomic absorption spectroscopy was used to determine Pb concentration in tissues. The results showed a 22.3 percentage point increase in Pb excretion ($P < 0.001$) and significant reductions in Pb accumulation in muscle (-47.2%), fat (-40%), and bone tissue (-48%). The survival rate increased by 5 percentage points ($P < 0.01$). These findings suggest that beespine biomass, due to its sorption and nutritional properties, can be considered a natural feed additive for reducing heavy metal accumulation and improving poultry health and productivity.

Keywords: heavy metals, melanin, melittin, lead, natural sorbents, poultry diet

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1. Introduction

The intensification of industry has led to the environmental pollution with various toxicants, among which heavy metals occupy an important place. Enterprises of the chemical, metallurgical, mining industry, as well as agricultural enterprises and motor vehicles are the main sources of environmental pollution with heavy metals in the world (Adamu et al., 2010).

It is known that the man-made load on the natural environment in Ukraine has decreased in the last few decades due to the reduction of industrial production in the chemical, metallurgical and mining industries. However, in some territories of Ukraine, it has been noted an increasing level of environmental pollution by various toxicants, in particular heavy metals, due to the intensification of agricultural production, which is characterized by a high level of chemicalization in crop production (Razanov et al., 2022; Razanov et al., 2023; Davydov et al., 2018).

Among the chemical substances used in crop production, mineral fertilizers, especially phosphoric and potassium are the most powerful source of soil pollution with heavy metals. The use of mineral fertilizers is increasing from year to year,

Pollution of the environment, especially agricultural soils with heavy metals, has caused certain problems in the production of high-quality plant material (Razanov et al., 2023). In particular, this has led to the accumulation of heavy metals in the grain of agricultural crops, which is used mainly for poultry feed.

The use of feed contaminated with heavy metals in feeding poultry leads to the accumulation of these toxicants in their body, as well as in their products. The use of such poultry products in the diet of the population increases the risk of accumulation of heavy metals in the human body, which can lead to diseases. It is known that the entry of heavy metals into living organisms, in particular, Pb, reduces immunity, antioxidant protection, enzyme activity, etc. (Satpathy et al., 2014; Han et al., 2016; El-Hassanin, 2020). The potential danger of the accumulation of heavy metals in poultry products is observed when poultry are kept in individual farms, where there is free access to natural minerals, which significantly increase the intake of heavy metals into the poultry's body and their production.

In order to reduce the intensity of accumulation of heavy metals in poultry products, sorbing feed additives are used in their feeding. In modern poultry farming, feed additives-sorbents are used in case of contamination of the poultry diet with heavy metals. Sorbents have high sorption properties that bind harmful substances, in particular, heavy metals and remove them from the body with undigested feed residues, which positively affects the quality of the products.

Feed additives-sorbents used in poultry farming mainly include zeolites, saponites, bentonites, which not only prevent the absorption of toxins in the gastrointestinal tract, but also enrich the diet of poultry with minerals (Razanov et al., 2018; Dydiv et. al., 2023). Along with preventing the absorption of heavy metals, sorbents increase enzymatic activity, digestibility and assimilation of nutrients in the diet.

The inclusion of natural sorbents in the diet of poultry contributes to the realization of their genetic potential, increases the intensity of the formation of live weight of poultry and the quality of the obtained products. The use of sorbents in feeding poultry, especially when they are kept freely on paddocks (characteristic of individual farms) is a necessary measure to improve the quality of products.

Our attention has been drawn to the use of biological mass of beespine as a feed additive of the sorption direction in the feeding of poultry. The biological mass of beespine is the bodies of bees that die in bee colonies during the winter period (waste of the beekeeping industry). The natural loss of bees in bee colonies during the winter period is generally up to 10%, and in some cases (due to violation of the conditions of their keeping during this period) it can be 40% or more. You can get from 300 to 600 g of this waste from one bee colony, which, as a rule, is not always used, but is buried (Kupchik et al., 2003; Oztokmak at al., 2023). It is known that beespine contains a number of useful components, in particular, bee venom, chitin, proteins, minerals, carbohydrates, etc. Chitin, which forms the upper shell of insects, including bees, contains from 20% to 30% of melanin. Melanin contains carbon, nitrogen, hydrogen, and sulfur, the amount of which can reach up to 12%. Due to the reduction of free radical oxidation, melanin has radioprotective properties (Kupchik at al., 2003; Razanov et al., 2020). It is known that melanin extracted from chitin of insects and waste from crab production absorbs ions of lead, zinc, copper, mercury and other heavy metals. It has been also determined that melanin isolated from the chitinous shell of bees has sorption properties. In addition, the biological mass of beespine contains up to 58.5% of proteins, 12.7% of fat, 13.5% of carbohydrates and up to 5.36% of minerals. 16 amino acids have been found in this biomass, including the highest amount of lysine, valine, glycine, proline, alanine, aspartic acid, as well as 18 mineral elements, among which sulfur, calcium, sodium, and phosphorus occupy the highest share.

Probably the interest of both scientists and practitioners is the content of beespine venom. It was revealed that the composition of bee venom includes peptides, enzymes, free amino acids, sugars, nitrogen, hydrogen, sulfur, carbon, calcium, phosphorus, copper, zinc, iodine, chlorine, amino acids, lipids the components of bee venom increase the protective functions of living organisms and have a high effect on the reduction of harmful microflora.

Peptides of bee venom increase the level of hemoglobin and reduce the content of cholesterol in the blood. In low doses, bee venom regulates fat and carbohydrate metabolism and increases resistance to radioactive irradiation (Razanov et al., 2018).

Bee venom is classified as a radioprotector by being injected into the body, it helps preserve stem cells, it has an anticoagulant effect. It has been established that when bee venom enters living organisms orally, i.e. through the digestive system, constituent elements of this raw material are found in their blood (Razanov et al., 2023).

The special value of bee venom is the content of 50% melittin polypeptide, which has a radioprotective, membrane-stabilizing, anti-inflammatory, anticoagulant effect.

Heparin, which has anti-inflammatory properties and belongs to anticoagulants, reducing the biosynthesis of thrombin, was also found in beespine. In addition, heparin activates the fibrolytic properties of blood to a certain extent and slightly lowers blood cholesterol.

Thus, the choice of bee submor biomass as a natural sorbent is due to its unique biochemical composition (submor contains chitin, melanin and melitin, biologically active substances with pronounced sorption, anti-inflammatory and detoxification properties). Unlike mineral sorbents (zeolites, bentonites), submor combines detoxifying effects with nutritional value, as it is rich in proteins, amino acids and minerals. This dual functional value gives grounds to consider it a promising component in poultry feeding under conditions of man-made stress (Kupchik et al., 2003; Pascual et al., 2019; Razanov et al., 2022).

To achieve the goal of our research, the task included the study of the development of young broiler chickens, their preservation of Pb balance and its accumulation in muscle, fat, cartilage, and bone tissue.

2. Materials and Methods

The research on the study of the set goal was carried out on broiler chickens of the Cob-500 breed. Experimental poultry for the research were selected according to the principle of groups-analogues, which are characterized by a common breed, age, weight, and keeping conditions (Kononenko et al., 2000). The broiler chickens of the control group were fed with a feed mixture (wheat grain – 65%, barley grain – 15%, corn grain – 15%, sunflower meal – 5%) during their breeding, while the poultry of the experimental group consumed 5% of the biological mass of beespine added to their feed mixture (95% of the feed mixture + 5% of the biological mass of beespine). The biological mass of beespine included crushed and dried bee bodies in the form of a powdered mass. Before feeding the powdered mass of beespine was subjected to a special treatment to increase the efficiency of assimilation by the poultry's body. The number of birds in each group was 50. The survival rate of young poultry fed with the biological mass of beespine was evaluated by their preservation from 5 to 45 days of age. In order to study the balance of Pb in the poultry's body after feeding them with the biological mass of beespine as part of feed mixture, a balance experiment was conducted, which involved the entry of Pb with feed into the body of poultry of the control and experimental groups and its removal with droppings. There were 10 birds in each group during the balance study. Experimental birds were kept in cage sections (Kozyr et al., 2022).

The biological mass of beespine had the powdered form, which was obtained as a result of drying the bodies of bees to remove 8% of moisture and grinding them. Prior to inclusion in the experimental diet, the powdered biological mass of beespine underwent standard safety evaluation. This included microbiological analysis (absence of Salmonella spp., E. coli, and mold contamination), heavy metal content (Pb), and moisture level control. All indicators were within acceptable safety limits according to international feed additive standards (FAO/WHO Codex Alimentarius). The drying process reduced microbial load.

The sample size (n=50 per group) was determined based on preliminary power analysis, aiming to detect a minimum difference of 5% in live weight with a power of 80% and $\alpha = 0.05$. Though the groups were limited in number, strict selection by analog pairs (breed, age, initial weight) and replication of measurements ensured adequate statistical reliability. Sample analysis was conducted in duplicate, and atomic absorption results were verified through internal quality control protocols (calibration with standard reference materials, blank controls, and recovery tests). The content of Pb in the feed mixture, muscles, fat and bones of broiler chickens was determined by the atomic absorption method (Methodical guidelines for atomic absorption methods for the determination of toxic elements in food products and food raw materials, 1992). The following tissues were used for the study: muscle (femoral), fat (abdominal) and bone (shin).

Biometric processing of the obtained research results was carried out taking into account the arithmetic mean value (M), the mean square deviation (m) and the reliability of the difference between the mean values (criteria P). Conventional symbols are used to show the probability in the tables: P < 0.05*; P < 0.01**; P < 0.001*** (Razanov, 2010; Field, 2017).

3. Results

The results of our research showed that the introduction of the biological mass of beespine in the feed mixture into the bird's body has a certain effect on the bird's live weight, its preservation and the balance of Pb in its body.

Table 1. Changes in the live weight of broiler chickens due to the introduction of beespine into their diet, g (n=4, M ± m)

Groups of experimental birds	Live weight of poultry on:							On average for the group
	1-day	7-day	14-day	21-day	28-day	35-day	42-day	
Control-1	40.1 ± 0.2	148 ± 2.4	462 ± 3.7	712 ± 4.3	1215 ± 4.1	1624 ± 5.0	2390 ± 7.3	2382 ± 143
Control-2	40.2 ± 0.3	146 ± 1.8	457 ± 1.9	708 ± 3.1	1221 ± 3.4	1704 ± 3.0	2310 ± 6.2	
Control-3	40.0 ± 0.1	152 ± 2.7	464 ± 3.1	713 ± 2.9	1208 ± 3.1	1720 ± 4.4	2402 ± 6.7	
Control-4	40.2 ± 0.2	151 ± 3.1	460 ± 2.8	720 ± 3.9	1220 ± 3.1	1672 ± 4.7	2430 ± 4.4	
Experiment-1	40.2 ± 0.2	147 ± 2.2	478 ± 2.6	718 ± 2.2	1221 ± 4.1	1763 ± 3.3	2630 ± 4.3	2511 ± 152
Experiment-2	40.1 ± 0.3	149 ± 2.4	465 ± 3.2	710 ± 3.2	1221 ± 3.5	1748 ± 3.2	2497 ± 3.2	
Experiment-3	40.0 ± 0.2	154 ± 3.2	470 ± 2.1	723 ± 3.0	1226 ± 3,2	1735 ± 4.1	2422 ± 4.7	
Experiment-4	40.2 ± 0.1	156 ± 2.3	464 ± 2.3	722 ± 2.7	1210 ± 4.1	1750 ± 3.1	2495 ± 5.6	

Analyzing the change in live weight of young broiler chickens, table. 1 for the introduction of the biological mass of beespine into their diet, it is necessary to note a certain tendency to increase this indicator in the birds of the experimental group, although no probable difference was found.

As evidenced by the research results of table. 1 live weight of broiler chickens at the end of the fattening period on the 42nd day in the control group was 2382 ± 43.2, and in the experimental group it was 2511 ± 152.2. That is, the live weight of the birds of the experimental group, whose diet was fed 5% of the biological weight of beespine, was higher compared to its counterparts in the control group.

Table 2. Average daily gain in live weight of broiler chickens, g (n=4, M ± m)

Groups of experimental birds	Live weight of poultry on:						On average for the group
	1-7 days	8-14-days	15-21 days	22-28 days	29-35 days	36-42 days	
Control-1	15.41	44.85	35.71	71.85	57.42	109.4	7.95 ± 0.97
Control-2	15.11	44.42	35.85	73.28	69.00	86.57	
Control-3	16.00	44.57	35.57	70.71	73.14	97.42	
Control-4	15.85	44.14	37.14	71.42	64.57	108.2	
Experiment-1	15.25	47.28	34.28	71.85	77.42	123.8	8.39 ± 0.73
Experiment-2	15.55	45.14	35.00	73.00	75.28	107.0	
Experiment-3	16.28	45.14	36.14	71.85	72.71	98.14	
Experiment-4	16.54	44.00	36.85	69.71	77.14	106.4	

Average daily weight gain of broiler chickens Table 2 was higher in the age periods of 1-7 days by 1.9%, 8-14 days - by 2.0%, 25-35 days - by 14.5% and from 36 to 42 days - by 8.0%. On average, over 42 days of the experimental period, the average daily increase in live weight of young broiler chickens increased by 5.5%.

Thus, the results of the balance of Pb in the body of broiler chickens fed with the biological mass of beespine as part of the main diet showed that the excretion of Pb with droppings in the poultry of the control group was within 45.5%, while it was 67.8% in the poultry of the experimental group. That is, the excretion of Pb from the body was higher by 22.3 p.p. in the poultry of the experimental group, compared to their analogues in the control group.

Table 3. Pb balance in the body of broiler chickens, mg (n=4, M ± m)

Groups of experimental birds	Intake with feed within a day		Removed with droppings per day		Retained in the body per day	
	mg	%	mg	%	mg	%
Control – I	0.079 ± 0.0047	100	0.037	46.8	0.042	53.2
Control – II	0.079 ± 0.0043	100	0.035	44.3	0.044	55.7
Control – III	0.079 ± 0.0025	100	0.035	44.3	0.044	55.7
Control – IV	0.079 ± 0.0032	100	0.037	46.8	0.042	53.2
Experiment – I	0.081 ± 0.0034	100	0.054	66.6	0.027	33.4
Experiment – II	0.081 ± 0.0027	100	0.057	70.3	0.024	29.7

Experiment – III	0.081 ± 0.0032	100	0.054	66.6	0.027	33.4
Experiment – IV	0.081 ± 0.0017	100	0.055	67.9	0.026	32.1

At the same time, it should be noted that Pb retention in the organs of poultry of the control group was 54.5%, while it was 32.2% or by 22.3 p.p. less than in their analogues of the experimental group. That is, when the biological mass of beespine was introduced into the diet of broiler chickens, it was observed an increase in the removal of Pb from their body and a decrease in its retention.

An increase in the level of Pb removal from the body of broiler chickens due to the consumption of biological mass of beespine as part of the main diet had a certain effect on the accumulation of this toxicant in its tissues (Table 3).

Table 4. Pb content in different tissues of broiler chickens, mg/kg (n=4, M ± m)

Researched tissues	Poultry of the control group	On average for the group	Poultry of the research group	On average for the group
Muscles	0.34	0.36 ± 0.014	0.19	0.19 ± 0.01***
	0.36		0.21	
	0.38		0.20	
	0.38		0.18	
Fat	0.21	0.20 ± 0.032	0.12	0.12 ± 0.021***
	0.19		0.13	
	0.22		0.10	
	0.21		0.13	
Bones	0.98	1.0 ± 0.018	0.52	0.52 ± 0.018***
	1.0		0.57	
	0.95		0.50	
	1.1		0.49	

According to the research results, the content of Pb was lower by 47.2% in the muscle tissue, by 40% in the fat tissue and by 48% in the bone tissue in the broiler chickens of the research group, compared to their analogues in the control group.

Table 5. Pb accumulation coefficient in different tissues of broiler chickens, mg/kg (n=4, M ± m)

Researched tissues	Poultry of the control group	Accumulation factor	Poultry of the research group	Accumulation factor

	Pb content in poultry' s diet	Pb content in poultry' s tissues		Pb content in poultry' s diet	Pb content in poultry/s tissues	
Muscles	0.79	0.36	0.45 ± 0.03	0.81	0.19	0.23 ± 0.037***
Fat	0.79	0.20	0.25 ± 0.012	0.81	0.12	0.14 ± 0.021***
Bones	0.79	1.0	1.26 ± 0.022	0.81	0.81	0.64 ± 0.04***

The results of studies on the coefficient of Pb accumulation in different tissues of broiler chickens (Table 5) showed that when they consumed the biological mass of beespine as part of the main diet, this indicator in the poultry of the experimental group was lower by 48.8% in muscles, by 44% in fat and by 49.2% in bones, compared to their analogues in the control group.

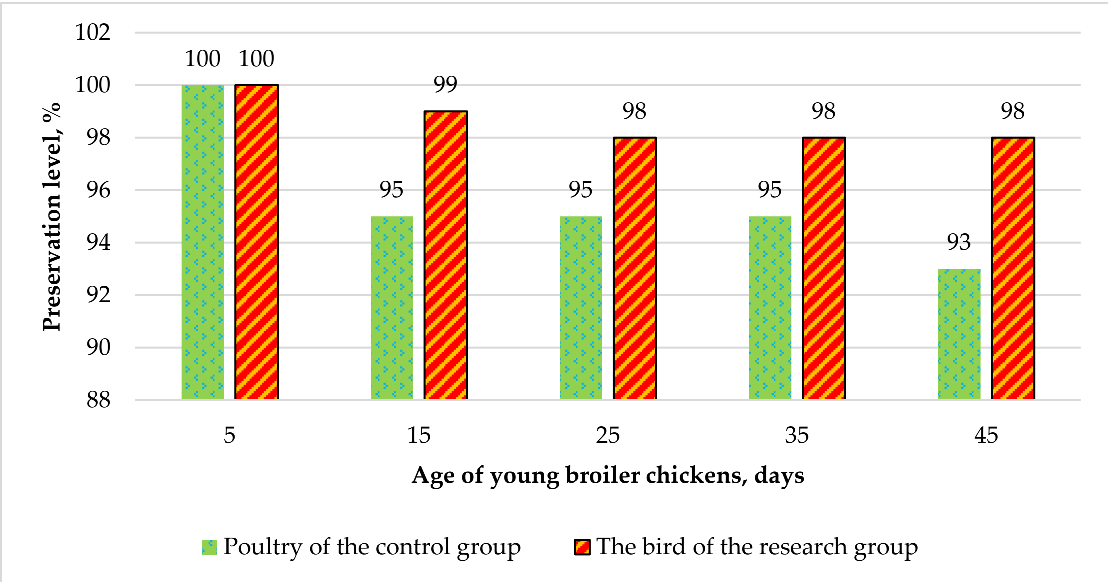

Fig. 1. The level of preservation of young broilers

The survival rate in poultry of the control group was 93%, while it was 98% in poultry of the experimental group, i.e. by 5 p.p. more. During the breeding period the loss of poultry in the control group was observed up to 25 days of age, while it was up to 15 days in the poultry of the experimental group. That is, the biological mass of beespine when entering the body of broiler chickens with compound feed contributed to a decrease in Pb absorption and an increase in the survival of poultry.

4. **Discussion**

It is known that the biological mass of beespine contains 58.45% protein, 12.67% fat, 13.52% non-nitrogenous extractives, and 5.36% ash – that is, the essential nutrients required for the functioning of living organisms, including poultry.

The results of our study demonstrated that the inclusion of 5% powdered beespine in the broiler chickens' diet led to a 5.4% increase in their live weight. However, this difference was not statistically significant when compared to the control group. In addition to the main nutrients, the powdered beespine also contains a range of biologically active substances and minerals. Among the amino acids, lysine, proline, alanine, valine, glycine, and aspartic acid were found in the highest concentrations. Furthermore, the presence of melittin – a chelated polypeptide – and melanin was confirmed. Among the mineral elements, the highest levels were recorded for sulfur (550 mg/100 g), calcium (440 mg/100 g), manganese (500 mg/100 g), and phosphorus (950 mg/100 g). Many of these biologically active compounds possess immunostimulatory properties, which are crucial for the development of stable immunity and resilience to environmental stressors in animals. Our findings revealed that the inclusion of 5% beespine biomass in the diet of broiler chickens significantly improved their survival rate by 5 percentage points ($P < 0.01$). The high nutritional and functional value of beespine is largely attributed to its bioactive substances – particularly melanin and melittin – which are responsible for its notable sorption activity. According to our results, the introduction of beespine biomass into the poultry diet resulted in a statistically significant decrease in lead (Pb) assimilation by 22.3 percentage points ($P < 0.001$). This was reflected in a marked reduction of Pb content in broiler femoral muscle tissue by 47.2% ($P < 0.001$), abdominal fat by 40% ($P < 0.001$), and tibial bone tissue by 48% ($P < 0.001$). Thus, the use of this beekeeping by-product – beespine – in the feeding of broiler chickens appears to be highly promising for improving bird survival and enhancing the safety and quality of poultry products.

5. Conclusions

According to the results of our research, feeding broiler chickens with the biological mass of beespine as part of the main diet (95% base feed + 5% beespine biomass) likely contributed to an increase in the excretion of Pb from the poultry's body by 22.3 p.p. ($P<0.001$), compared to the control group that did not receive the biomass, which indicates the preservation of its sorption properties. The increased excretion of Pb through droppings had a measurable effect on the content of this toxicant in poultry tissues. Specifically, when the diet included 5% beespine biomass, Pb concentrations likely decreased by 47.2% ($P<0.001$) in femoral muscles, by 40% ($P<0.001$) in abdominal fat, and by 48% ($P<0.001$) in tibial bone tissue. In addition, the survival rate of broiler chickens improved by 5 p.p. as a result of incorporating beespine biomass into their diet. At the same time, no significant difference in live body weight was observed between the experimental and control groups.

Therefore, the observed reduction in Pb accumulation in broiler tissues following the administration of beespine biomass highlights the effective binding properties of its melanin- and chitin-based compounds. In contrast to conventional mineral sorbents, the organic matrix of beespine may facilitate enhanced interaction with toxicants at the intestinal level. Additionally, the presence of immunoactive peptides such as melittin may support improved systemic resistance, as reflected in increased survival rates. This dual function – detoxification and nutritional enrichment – positions beespine biomass as a novel and sustainable solution for improving food safety in poultry production.

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